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(54) Title: NOVEL 1α-HYDROXY VITAMIN D_4 AND NOVEL INTERMEDIATES AND ANALOGUES

(57) Abstract

Novel 1α-hydroxy vitamin D_4 and novel analogues, 1,25 dihydroxy vitamin D_4 and 1,24 dihydroxy vitamin D_4 which are useful as active compounds of pharmaceutical compositions for the treatment of disorders of calcium metabolism. Preparation of the novel 1α-hydroxy vitamin D_4 starts from ergosterol which is converted in six steps to 22,23-dihydroergosterol. 22,23-dihydroergosterol was irradiated to yield vitamin D_4 which is converted in four steps to 1α-hydroxy vitamin D_4 using a cyclovitamin procedure which produces the novel intermediates, vitamin D_4 tosylate, 3,5 cyclovitamin D_4 and 1α-hydroxy cyclovitamin D_4. 1,25 dihydroxy vitamin D_4 and 1,24 dihydroxy vitamin D_4 are isolated as biological products of the metabolism of novel 1α-hydroxy vitamin D_4 using cultured human liver cells.

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NOVEL 1α-HYDROXY VITAMIN D_4 AND NOVEL INTERMEDIATES AND ANALOGUES

TECHNICAL FIELD

This invention relates to biologically active vitamin D_4 compounds. More specifically, this invention relates to novel 1α-hydroxy vitamin D_4 and novel intermediates used in its synthesis, novel 1,25 dihydroxy vitamin D_4, and novel 1,24 dihydroxy vitamin D_4.

This invention also relates to a pharmaceutical composition which includes a pharmaceutically effective amount of the novel 1α-hydroxy vitamin D_4 compounds, and to a method of controlling abnormal calcium metabolism by administering a pharmaceutically effective amount of the novel compounds.

BACKGROUND

Vitamin D is known to be important in the regulation of calcium metabolism in animals and man. See, Harrison's Principals of Internal Medicine: Part Eleven, "Disorders of Bone and Mineral Metabolism, Chapter 335," E. Braunwald, et al., (eds.), McGraw-Hill, New York, 1987, pp. 1860-1865. The two most commonly known, useful forms of vitamin D are vitamin D_3 and vitamin D_2. Vitamin D_3 is synthesized endogenously in the skin of animals and man, whereas vitamin D_2 is the form of vitamin D supplied by plants. Vitamin D_2 differs from vitamin D_3 in that it contains a double bond between C22 and C23 and further contains a C24-methyl group. In man and rats, vitamin D_3 and vitamin D_2 have equivalent biopotency.

Vitamin D_4, also known as irradiated 22,23-dihydro-ergosterol or 22,23-dihydro vitamin D_2 or 22,23-dihydroergocalciferol, differs from vitamin D_3 in that it contains a C24 methyl group. Vitamin D_4 was first described in 1936. See, Grab, W., Z.Physiol. Chem., 243:63 (1936); McDonald, F.G., J. Biol. Chem., 114:IVX (1936). See also, Windaus, A. and Trautmann, G., Z. Physiol. Chem., 247:185-188 (1937). These references report some disagreement as to the level of biological activity of the vitamin suggesting that in the rat, vitamin D_4 is one-third or three-fourths as active as vitamin D_3

therapeutic agent.

SUMMARY OF THE INVENTION

The novel compounds of the invention, 1α-hydroxy vitamin D_4, 1,25-dihydroxy vitamin D_4 and 1,24-dihydroxy vitamin D_4, are bioactive forms of vitamin D_4. The present inventors have discovered that these active forms of vitamin D_4 display much greater biopotency than would be predicted on the basis of the previously reported bioassays of vitamin D_4. The present inventors have also discovered, that the bioactive novel compounds are less toxic than would be predicted on the basis of their biopotency. This combination of high activity with low toxicity makes the compounds of the invention useful as therapeutic agents in the treatment of disorders of calcium metabolism. The novel compounds of the invention are advantageously used as the active compounds of pharmaceutical compositions for diseases induced by abnormal metabolism of calcium.

In order to study the novel compounds of the invention, it was necessary to develop processes for their production. One alpha-hydroxy vitamin D_4 was made synthetically and in the course of that synthesis, novel intermediates were also produced. 1,25-dihydroxy vitamin D_4 and 1,24-dihydroxy vitamin D_4 are isolated as biological products of the metabolism of 1α-hydroxy vitamin D_4.

Other advantages and a fuller appreciation of the specific adaptations, compositional variations, and physical and chemical attributes of the present invention will be gained upon an examination of the following detailed description of the invention, taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will hereinafter be described in conjunction with the appended drawings, wherein like designations refer to like elements throughout and in which:

Figure 1 illustrates preparative steps for the synthesis of vitamin D_4; and

Figure 2 illustrates preparative steps for the synthesis of 1α-hydroxy vitamin D_4 starting with vitamin D_4.

four-step process to yield 1α-hydroxy vitamin D_4 using a procedure similar to that described by Paaren, et al., J. Org. Chem., 1980, 45:3253.

Specifically, ergosterol is acetylated to form the 3β-acetate. This ergosterol acetate is subjected to hydroxyhalogenation at the 5,6 double bond to form the 6α-chloro-5α-hydroxy derivative. This chlorohydrin is reduced and reacetylated to the 5α-hydroxy (i.e., 5α-ol) derivative. The 5α-ol is subjected to hydrogenation to saturate the side chain. The resulting 3β-acetoxyergost-7en-5α-ol is reduced to 22,23 dehydroergosterol acetate which is in turn reduced to yield 22,23 dehydroergosterol. The 22,23 dehydroergosterol is then irradiated to form vitamin D_4. Vitamin D_4 is then tosylated to yield 3β-tosyl vitamin D_4. The tosylate is displaced by solvolysis to yield the 6-methoxy-3,5-cyclovitamin D_4. The cyclovitamin D_4 is subjected to allyllic oxidation to form the 1α-hydroxy cyclovitamin derivative. The 1α-hydroxy cyclovitamin derivative is sequentially solvolyzed and subjected to a Diels-Alder-type reaction which removes the 5-methoxy group and separates the 1α-hydroxy vitamin D_4 (5,6-cis) from the 5,6 trans-1α-hydroxy vitamin D_4.

The 1,24 dihydroxy vitamin D_4 and 1,25 dihydroxy vitamin D_4 metabolites of 1α-hydroxy vitamin D_4, are synthesized by incubating the 1α-hydroxy derivatives with human liver cells, culturing the cells, and recovering the 1,24 dihydroxy or 1,25 dihydroxy vitamin D_4. Using vitamin D receptor protein binding tests, these metabolites are determined to be biologically active.

The compounds of formula (I) have been found to possess valuable pharmacological activity, namely, as controlling agents for calcium metabolism, especially serum calcium concentrations. Specifically, the compounds of formula (I) increase serum calcium concentrations in rats with vitamin D deficiency. It has also been found that the compounds of formula (I) have low toxicity, which enhances their pharmaceutical properties. Compounds of formula (I) have a toxicity, as measured by the LD_{50} test, which is similar to that of corresponding vitamin D_2 compounds and lower than that of corresponding vitamin D_3 compounds. Thus, the compounds of the invention are applicable

vegetable oils (e.g., corn oil, cottonseed oil, peanut oil, olive oil, coconut oil), fish liver oils, oily esters such as Polysorbate 80, polyethylene glycols, gelatine, carbohydrates (e.g., lactose, amylose or starch), magnesium stearate, talc, silicic acid, viscous paraffin, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, hydroxy methylcellulose, polyvinyl pyrrolidone, etc.

The pharmaceutical preparations can be sterilized and, if desired, be mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or one or more other active compounds, for example, vitamin D_3 or D2 and their 1α-hydroxylated metabolites, conjugated estrogens or their equivalents, anti-estrogens, calcitonin, biphosphonates, calcium supplements, cobalomin, pertussis toxin and boron.

For parenteral application, particularly suitable are injectable, sterile solutions, preferably oily or aqueous solution, as well as suspensions, emulsions, or implants, including suppositories. Ampoules are convenient unit dosages.

For enteral application, particularly suitable are tablets, dragees, liquids, drops, suppositories, lozenges, powders, or capsules. A syrup, elixir, or the like can be used if a sweetened vehicle is desired.

Sustained or directed release compositions can also be formulated, e.g., liposomes or those in which the active compound is protected with differentially degradable coatings, e.g., by microencapsulation, multiple coatings, etc.

For topical application, suitable nonsprayable viscous, semi-solid or solid forms can be employed which include a carrier compatible with topical application and having a dynamic viscosity preferably greater than water. Suitable formulations include, but are not limited to, solutions, suspensions, emulsions, creams, ointments, powders, liniments, salves, aerosols, transdermal patches, etc., which are, if desired, sterilized or mixed with auxiliary agents, e.g., preservatives, stabilizers, demulsifiers, wetting agents, etc.

For rectal administration, compounds are formed into a pharmaceutical composition containing a suppository base such as

3000 Computer in $CDCl_3$ solutions with $CHCl_3$ as an internal standard. Infrared spectra were recorded with a Fourier transform (FTIR) using samples as potassium bromide (KBr) pellets or as liquids. Mass spectra were recorded with a Finnigan MAT-90 mass spectrometer at 20 eV/CI. Melting points are determined on a Hoover-Thomas (capillary) Uni-Melt and a Fisher-Johns melting point apparatus (cover-slip type).

Example 1: Synthesis of 1α-hydroxy vitamin D_4

Ergosterol (II) was converted to ergosterol acetate (III) by dissolving 100 g (0.25 mol) ergosterol in 600 ml of anhydrous pyridine and 68 ml (0.7 mol) acetic anhydride. The solution was stirred overnight at room temperature after which time the solution was cooled by adding 1.2 L ice, causing a precipitate to form. The precipitate was washed five times with 400 ml portions of water, then once with 400 ml of CH_3CN. The resulting product was air dried to yield 79 g (71%) of ergosterol acetate as a white crystalline solid and had the following characteristics: melting point (m.p.): 169-171°C; ^1H NMR: (400 MHz, $CDCl_3$), δppm 2.05 (3H, s, 3β-CH_3CO), 4.65-4.75 (1H, m, 3α-H) 5.15-5.25 (2H, m, 22-H and 23-H), 5.4 (1H, d, 6-H), 5.6 (1H, d, 7-H); FTIR [KBr]: 1734 cm^{-1} (C=O stretching) 968 cm^{-1} (C-H bending).

Ergosterol acetate (III) (26 gm, 0.062 M) was dissolved in 2.5 L of freshly distilled deoxygenated toluene. To this solution 9 ml (0.111 mol) chromyl chloride dissolved in 240 ml dry CH_2Cl_2 was added under nitrogen at -78°C over a thirty minute period. The reaction system was stirred at -78°C for an additional fifteen minutes, and then 62 ml of a saturated solution of sodium borohydride in ethanol was added in one portion. After stirring at -78°C for an additional fifteen minutes, the reaction solution was poured into a two phase system of 3N hydrochloric acid (3L) and benzene (3L). The organic layer was separated, then washed with water (2L), twice with a brine solution (2 x 1L) and then dried with anhydrous $MgSO_4$. The dried solution was filtered and concentrated in vacuo. The crude crystalline product was then treated with CH_3CN (280ml) and filtration of the thus formed slurry yielded 12.5 g (41%) of white crystalline 3β-Acetoxy-6α-chloroergosta-7,22-dien-5α-ol

was converted to a slurry with CH_3CN (100 ml). The product was collected by filtration and recrystallized from CH_3CN to yield 4.5 g. (39%) of a white crystalline 22,23-dihydroergosteryl acetate (VII) and had the following characteristics: m.p.: 144-147°C; ^1H NMR: (400 MHz, $CDCl_3$), δppm 2.05 (3H, s, 3β-OAc), 4.65-4.75 (1H, m, 3α-H), 5.4 (1H, d, 6-H), 5.6 (1H, d, 7-H); FTIR [KBr]: 1734 cm^{-1} (C=O stretching).

22,23-dihydroergosteryl acetate (VII) (4.8 g, 0.011 mol) was added at once to a stirred suspension of lithium aluminium hydride (2.5 g, 0.066 mol) in dry ether (1.1 L) at room temperature. The mixture was stirred for two hours at room temperature. 5N NaOH was added to destroy excess lithium aluminium hydride and H_2O (500 ml) was then added. The aqueous solution was then extracted with four 250 ml portions of ether. The combined ether extracts and combined organic layer were washed with brine solution (1 L), then dried with Na_2SO_4. Evaporation of ether under reduced pressure gave the compound, 22,23-dihydroergosterol, (VIII) (4.1 g, 94%) as a white crystalline material and had the following characteristics: m.p.: 147-150°C; ^1H NMR: (400 MHz, $CDCl_3$), δppm 3.6-3.7 (1H, m, 3α-H), 5.4 (1H, d, 6H), 5.6 (1H, d, 7-H); FTIR [KBr]: 3400 cm^{-1} (O-H stretching).

22,23-dihydroergosterol (VIII) (2.0 g, 5.0 mmol) was dissolved in a solution of diethyl ether and benzene (4:1, 600 ml) and irradiated (Hannovia immersion lamp, 450 watts) with stirring under argon in a water-cooled quartz vessel for three hours. The solution was concentrated *in vacuo* to yield a gummy solid, which was redissolved in 100 ml. of ethanol and heated at reflux under argon for eight hours. Then, the solution was concentrated *in vacuo* and the residue was adsorbed on a silica gel column and eluted with 30% ethyl acetate in hexane to afford vitamin D_4 (22,23-dihydroergocalciferol) (IX) with a yield of 1.2 g. (60%) and with the following characteristics: ^1H NMR: (400 MHz, $CDCl_3$), δppm 0.55 (3H, s, 18-H_3) 0.78 (6H, dd, 26-H_3 and 27-H_3) 0.87 (3H, d, 21-H_3) 0.93 (3H, d, 28-H_3) 3.94 (1H, m, 3-H) 4.82 (1H, m (sharp), 19-H), 5.04 (1H, m (sharp), 19-H), 6.04 (1H, d, 7-H) 6.24 (1H, d, 6-H).

To a stirred solution of vitamin D_4 (IX) (3.0 g, 7.5 mmol) in 10 ml of dry pyridine was added freshly recrystallized p-

saturated NaCl solution (2 x 200 ml), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was absorbed on a silica gel column and eluted with 30% ethyl acetate in hexane to afford 0.45 g. (29%) of the novel intermediate compound 1α-hydroxy 3,5-cyclovitamin D_4 (XII) (oil) and had the following characteristics: ^1H NMR (400 MHz, $CDCl_3$), δppm 0.54 (3H, s, 18-H_3) 0.78 (6H, dd, 26-H_3 and 27-H_3) 0.86 (3H, d, 21-H_3) 0.95 (3H, d, 28-H_3) 3.26 (3H, s, OCH_3) 4.2 (1H, d, 6-H), 4.22 (1H, m, 1-H), 4.95 (1H, d 7-H), 5.18 (1H, d, 19-H) 5.25 (1H, d, 19-H).

A solution of 1α-hydroxy 3,5-cyclovitamin D_4 (XII) (0.45 g, 1.05 mmol) in a solution of dimethyl sulfoxide (4.5 ml) and glacial acetic acid (3.6 ml) was heated to 50°C under argon for one hour. The reaction mixture was then poured over ice and saturated $NaHCO_3$ solution (100 ml), and extracted with ether (3 x 200 ml). The combined ether extracts were washed with saturated $NaHCO_3$ solution (3 x 200ml), water (3 x 200 ml) and saturated NaCl solution (3 x 200 ml), dried over $MgSO_4$, concentrated *in vacuo* to give a mixture containing 5,6-cis and 5,6-trans 1α-hydroxy vitamin D_4 (about 4:1 by ^1H NMR) with a yield of 0.4g, (92%). The mixture of 5,6-cis and 5,6-trans 1α-hydroxy vitamin D_4 (0.4 g, 0.97 mmol) was dissolved in ethyl acetate (25 ml) and treated with freshly recrystallized maleic anhydride (0.08 g, 0.8 mmol). This reaction mixture was heated to 35°C under argon for 24 hours. After evaporation of the solvent *in vacuo*, the crude mixture was chromatographed over a silica gel column using ethyl acetate and hexane (1:1) as eluent, to afford the novel active form of vitamin D_4, 5,6-cis 1α-hydroxy vitamin D_4 (XIII) with a yield of 90 mg (23%) and had the following characteristics: m.p.: 128-130°C; IR ν_{max} (Neat): 3400 cm^{-1} (OH stretching); ^1H NMR (400 MHz, $CDCl_3$), δppm 0.55 (3H, s, 18-H) 0.79 (6H, dd, 26-H_3 and 27-H_3) 0.87 (3H, d, 21-H_3) 0.94 (3H, d, 28-H_3), 4.24 (1H, m, 3-H), 4.44 (1H, m, 1-H), 5.02 (1H, m (sharp), 19-H), 5.34 (1H, m (sharp), 19-H), 6.02 (1H, d 7-H), 6.4 (1H, d, 6-H); Mass spectrum [CI] m/e (relative intensity): 415 (M+1, 41%) 397, (M+1-OH 100%), 379 (27%), 135 (22%).

Example 2: Biological testing of 1α-hydroxy vitamin D_4

Male weanling rats (Holtzman strain, Holtzman Company, Madison, Wisconsin) were fed a vitamin D deficient diet

method. Rats were fed a standard laboratory diet for 8-10 weeks. Five animals of each sex were administered one oral dose of 1α-OH-D_4. The animals were observed for 14 days, and the number of deaths noted. The LD_{50} value was determined to be about 1.0 mg/kg in males and 3.0 mg/kg in females.

For comparison, the LD_{50} value for 1α-hydroxy vitamin D_2 under the same conditions was found by applicant's to be 1.7 and 1.8 mg/kg. in male and female rats, respectively. The toxicity of 1α-hydroxy vitamin D_2 has previously been reported as less than 1α-hydroxy vitamin D_3. Sjoden, G., Smith, C., Lindgren, U., and DeLuca, H.F., Proc. Soc. Experimental Biol. Med., 178:432-436 (1985).

Example 4: Generation and Isolation of 1,25-dihydroxy vitamin D_4

The 1α-hydroxy vitamin D_4 of the present invention is incubated with cultured human liver cells which metabolize the compound to several products including the metabolite 1,25 dihydroxy vitamin D_4. The 1,25 metabolite is isolated and purified by high pressure liquid chromatography and identified by gas-chromatography-mass spectrometry. Binding studies demonstrate that the 1,25 dihydroxy vitamin D_4 has good binding affinity for the mammalian vitamin D receptor protein indicating it is biologically active. The procedures used are similar to that described by Strugnell, et. al., Biochem. Pharm. Vol. 40:333-341 (1990).

Example 5: Generation and isolation of 1,24-dihydroxy vitamin D_4

Generation and isolation of 1,24 dihydroxy vitamin D_4 is accomplished as described in Example 4, above. The 1α-hydroxy vitamin D_4 of the present invention is incubated with cultured human liver cells which metabolize the compound to several products including the metabolite 1,24 dihydroxy vitamin D_4. The 1,24 metabolite is isolated and purified using high pressure liquid chromatography and identified by gas-chromatography-mass spectrometry. Binding studies with the new metabolite demonstrate that the metabolite has good binding affinity for the mammalian vitamin D receptor protein which indicates the drug is biologically active.

comparisons of urinary hydroxyproline excretion, serum and urine calcium levels, creatinine clearance, blood urea nitrogen, and other routine determinations.

This study demonstrates that patients treated with 1α-vitamin D_4 exhibit significantly higher total body, radial, femoral and/or spinal bone densities relative to patients treated with placebo. The treated patients also exhibit significant elevations in serum osteocalcin. Bone biopsies from the treated patients show that 1α-vitamin D_4 stimulates normal bone formation. The monitored safety parameters confirm an insignificant incidence of hypercalcemia or hypercalciuria, or any other metabolic disturbance with 1α-vitamin D_4 therapy.

Example 9:

A clinical study is conducted with healthy postmenopausal women having ages between 55 and 60 years. The study involves up to 80 patients randomly divided into two treatment groups, and continues for 12 to 24 months. One treatment group receives a constant dosage of 1α-vitamin D_4 (u.i.d.; a dose level above 3.0 μg/day) and the other receives a matching placebo. The study is conducted as indicated in Example 2 above.

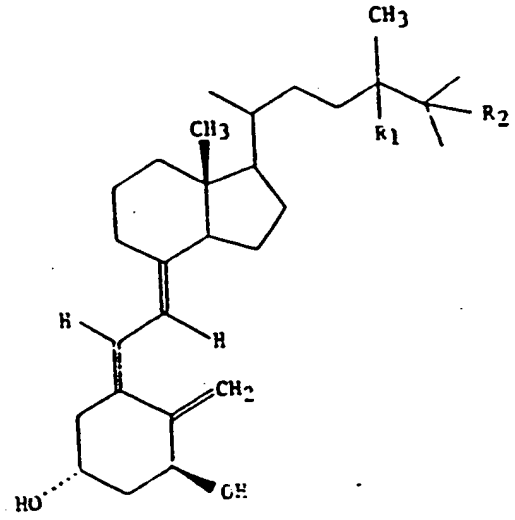
This study demonstrates that patients treated with 1α-vitamin D_4 exhibit reduced losses in total body, radial, femoral and/or spinal bone densities relative to baseline values. In contrast, patients treated with placebo show significant losses in these parameters relative to baseline values. The monitored safety parameters confirm the safety of long-term 1α-vitamin D_4 administration at this dose level.

Example 10:

A twelve-month double-blind placebo-controlled clinical trial is conducted with thirty men and/or women with renal disease who are undergoing chronic hemodialysis. All patients enter an eight-week control period during which time they receive a maintenance dose of vitamin D_3 (400 IU/day). After this control period, the patients are randomized into two treatment groups: one group receives a constant dosage of 1α-vitamin D_4 (u.i.d.; a dosage greater than 3.0 μg/day) and the other group receives a matching placebo. Both treatment groups

CLAIMS:

1. The compound of the formula (I):



(I)

wherein R_1 is either H or OH and R_2 is either H or OH and salts, hydrates and solvates thereof.

2. The compound of claim 1, wherein said compound is 1α-hydroxy vitamin D_4.

3. The compound of claim 1, wherein said compound is 1,24 dihydroxy vitamin D_4.

4. The compound of claim 1, wherein said compound is 1,25 dihydroxy vitamin D_4.

5. The compound of claim 1, wherein said compound is biologically active.

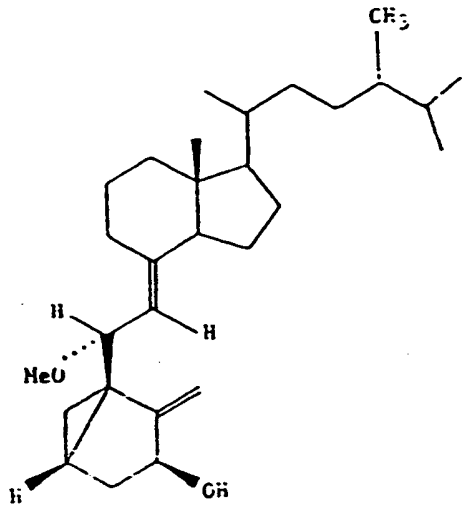
6. The compound of formula (I) according to claim 1, wherein R_1 is H or OH and R_2 is H or OH and wherein said compound exhibits biological activity approaching that of 1,25 vitamin D_3 and wherein said compound is less toxic than 1α-hydroxy vitamin D_3 as determined by comparative LD_{50} values in rats.

7. The compound of claim 6, wherein said compound is 1α-hydroxy vitamin D_4.

8. The compound of claim 6, wherein said compound is 1,25 dihydroxy vitamin D_4.

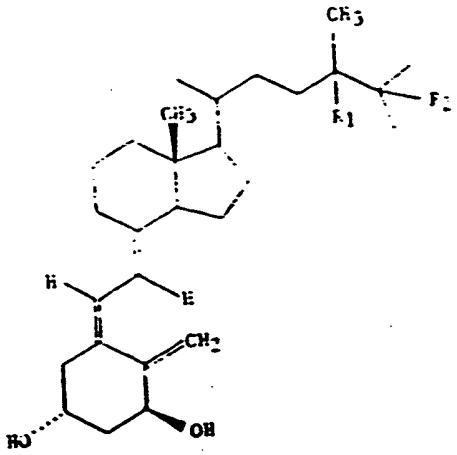
9. The compound of claim 6, wherein said compound is 1,24 dihydroxy vitamin D_4.

12. The 1α-hydroxy 3,5 cyclovitamin D_4 of the formula (XII):



(XII)

13. A pharmaceutical composition, comprising an amount effective to increase serum calcium in a patient suffering vitamin D deficiency of a compound of the formula (I):



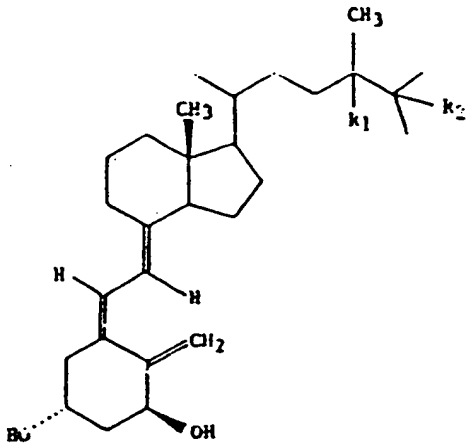
(I)

wherein R1 is either H or OH and R2 is either H or OH in combination with a pharmaceutically acceptable vehicle.

14. The pharamaceutical composition of claim 13, wherein said amount is administered orally.

15. A method of treating vitamin D deficiency induced diseases comprising administering to a patient suffering

increase serum calcium in the mammal, of a compound having the formula (I):



(I)

wherein R1 is either H or OH and R2 is either H or OH.

18. The method of claim 17, wherein said mammal suffers a vitamin D deficiency.

19. The method of claim 17, wherein said compound is administered in a daily dose of about 0.04 µg to about 1.5 µg per kg of body weight of the treated mammal.

20. The method of claim 17, wherein the hypocalcemia is vitamin D dependent rickets, hypoparathyroidism, post-operative renal osteodystrophy, liver cirrhosis, or steatorrhoea.

21. A method of producing vitamin D_4 tosylate, comprising reacting vitamin D_4 with toluenesulfonyl chloride in the presence of dry pryridine.

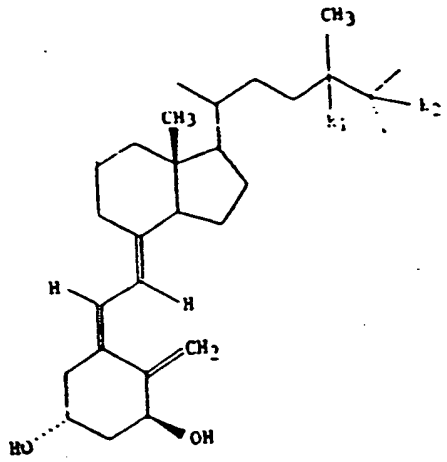
22. A method of producing 3,5 cyclovitamin D_4, comprising subjecting vitamin D_4 tosylate to buffered solvolysis.

23. A method of producing 1α-hydroxy 3,5 cyclovitamin D_4, comprising allylically oxidizing the 3,5 cyclovitamin D_4 with selenium dioxide.

24. A method of producing 1α-hydroxy vitamin D_4, comprising solvolizing the 1α-hydroxy 3,5 cyclovitamin D_4 with a mixture of dimethylsulfoxide and an organic acid to form an admixture of the 5,6 cis 1α-hydroxy and 5,6 trans 1α-hydroxy vitamin D_4 and subjecting the admixture to a Diels-Alder reaction forming an adduct of the 5,6 trans 1α-hydroxy vitamin D_4 to yield the 1α-hydroxy vitamin D_4.

25. A method of producing 1α-hydroxy vitamin D_4,

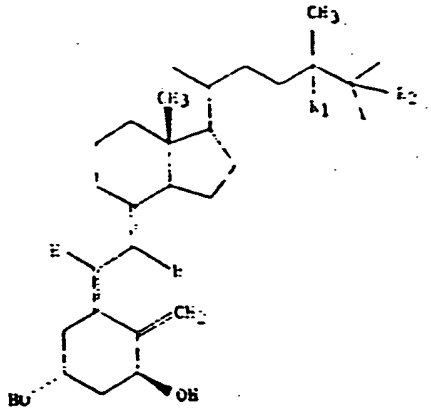
an effective amount of at least one compound of formula (I):



(I)

wherein R1 is either H or OH and R2 is either H or OH.

28. A prophylactic or therapeutic pharmaceutical composition for vitamin D deficient diseases, comprising a physiologically acceptable vehicle and an effective amount of at least one compound of formula (I):

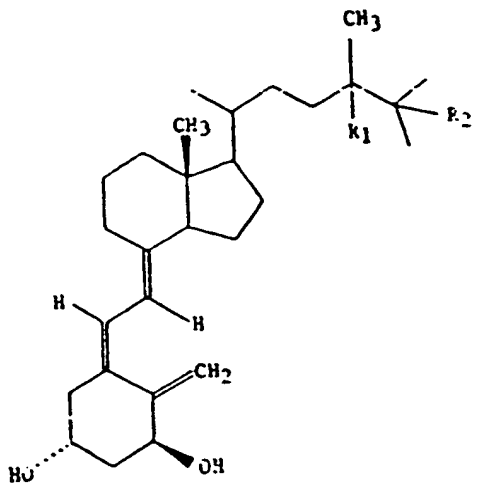


(I)

wherein R1 is either H or OH and R2 is either H or OH.

29. A method of controlling calcium metabolism in a mammal, comprising administering to a mammal a pharmaceutically

formula (I):



(I)

wherein R_1 is either H or OH and R_2 is either H or OH in combination with a pharmaceutically acceptable vehicle.

34. A method for treating vitamin D deficiency-induced hypocalcemia, comprising:

(a) reducing ergosterol, under such conditions and in sufficient quantity to produce 22,23 dihydroergosterol;

(b) irradiating the 22,23 dihydroergosterol to produce vitamin D_4;

(c) hydroxylating the vitamin D_4 under such conditions and in sufficient quantity to produce 1α-hydroxy vitamin D_4;

(d) purifying the vitamin D_4; and

(e) administering to a mammal suffering from vitamin D deficiency-induced hypocalcemia an amount effective to increase serum calcium of 1α-hydroxy vitamin D_4 in admixture with a pharmaceutically acceptable vehicle.

35. A pharmaceutical composition for treating osteoporosis comprising a physiologically acceptable vehicle and an effective

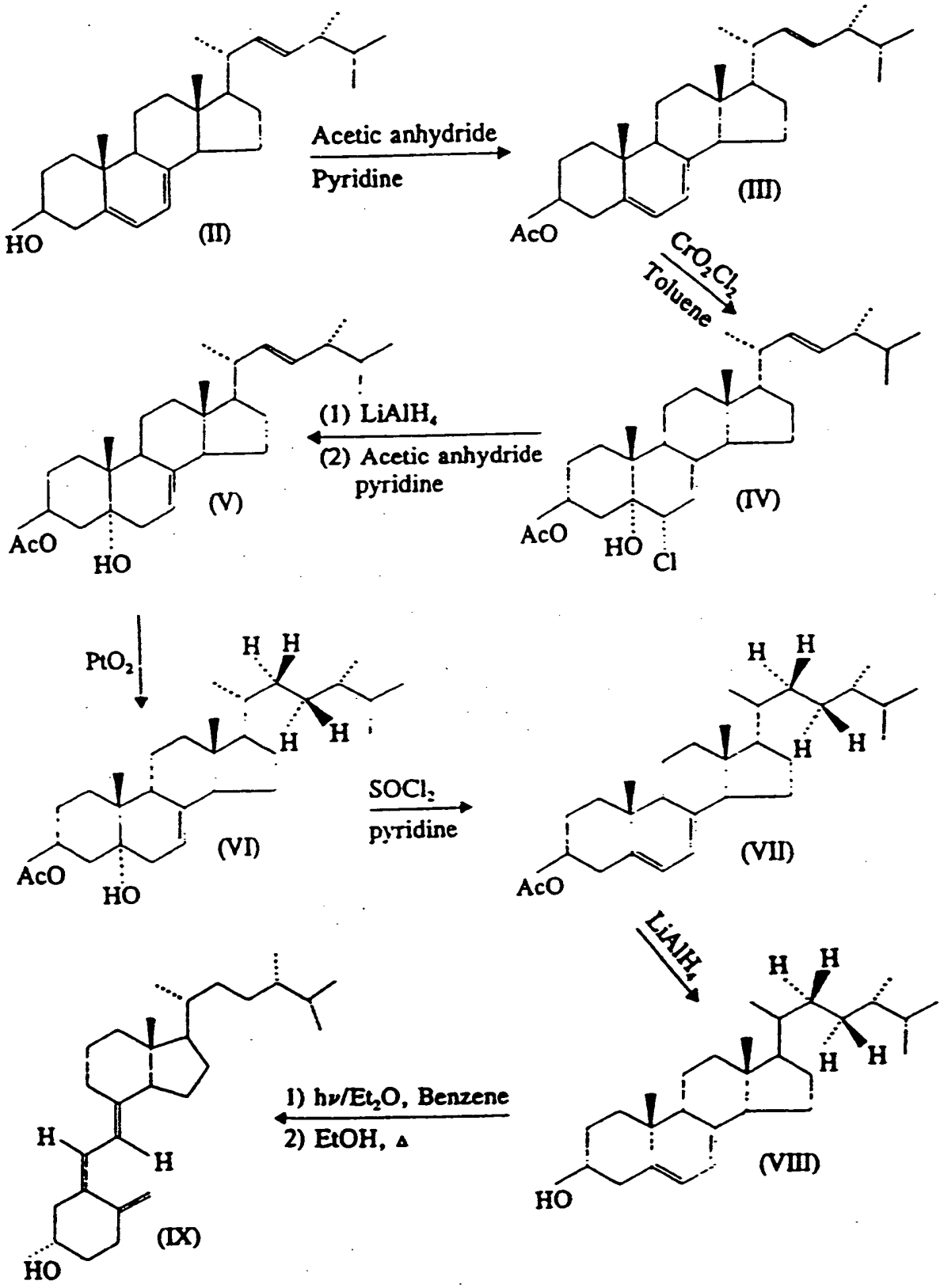

Acetic anhydride
Pyridine
(II)
(III)
HO
AcO
CrO_2Cl_2
Toluene
(1) $LiAlH_4$
(2) Acetic anhydride
pyridine
(IV)
(V)
AcO
HO
Cl
PtO_2
$SOCl_2$
pyridine
(VI)
(VII)
$LiAlH_4$
1) $h\nu/Et_2O$, Benzene
2) EtOH, Δ
(VIII)
(IX)

FIGURE 1

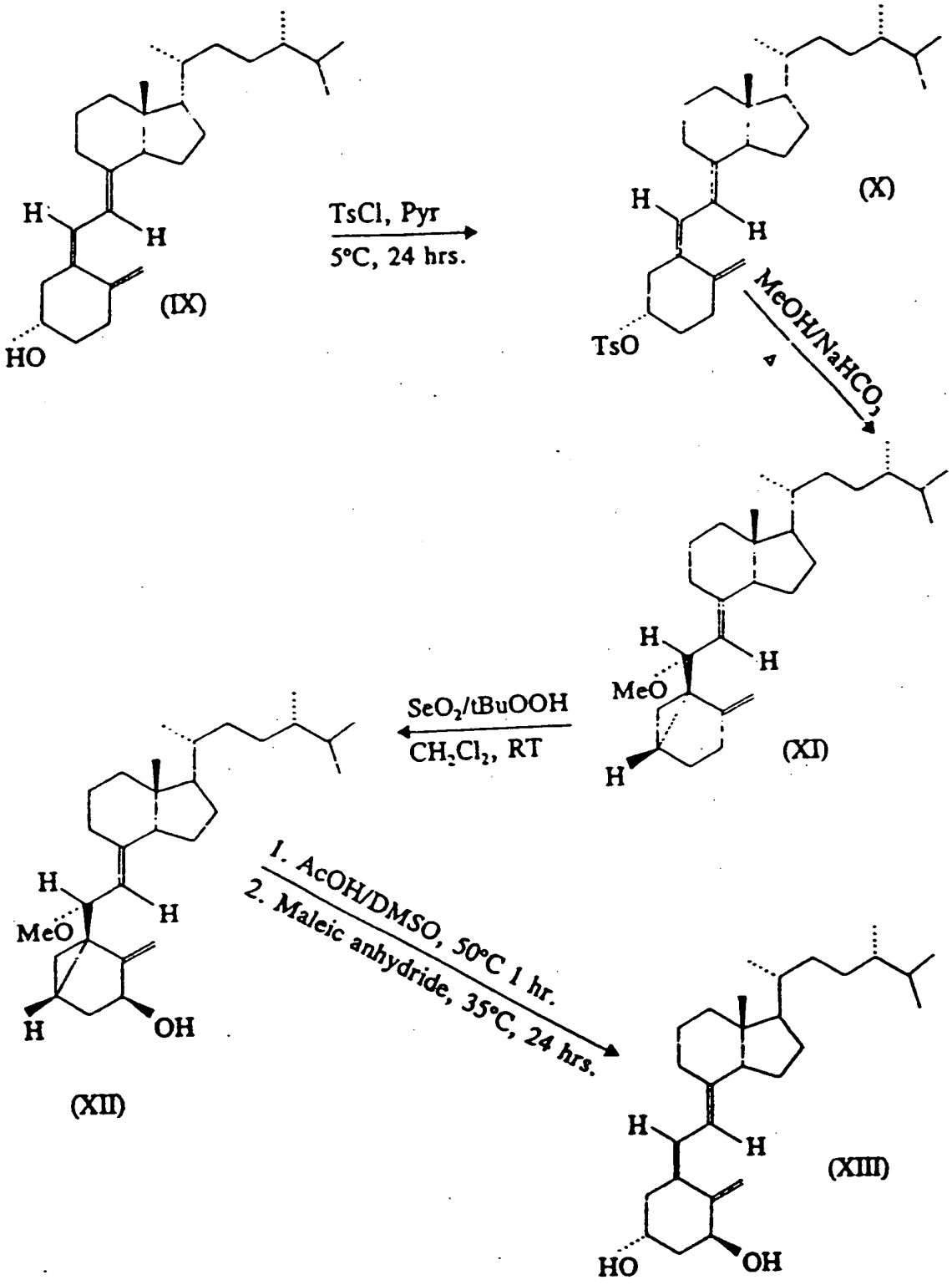

TsCl, Pyr
5°C, 24 hrs.
(IX)
(X)
MeOH/NaHCO3
(XI)
SeO2/tBuOOH
CH2Cl2, RT
1. AcOH/DMSO, 50°C 1 hr.
2. Maleic anhydride, 35°C, 24 hrs.
(XII)
(XIII)

FIGURE 2

INTERNATIONAL SEARCH REPORT

International Application No: PCT/US91/06865

I. CLASSIFICATION OF SUBJECT MATTER

IPC(5): C07C 9/00, A61K 31/59
US CL 552/653, 514/168

II. FIELDS SEARCHED

Minimum Documentation Searched

Classification System	Classification Symbols
U.S.	552/653, 514/168

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched

III. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	US, A, 4,202,859 (DULUCA ET AL.) 13 MAY 1980	1-9,13-15, 17-20,27-31, 33-36
Y	DELUCA ET AL. Arch. Biochem. and biophys. 124, 122-128 (1965) Synthesis, Biological Activity and Metabolism of 22,23 ^{3}H Vitamin D_4.	1-9,13-15,17-20,27-31,33-36
Y	Windaus, et al. Z. Physiol. Chem. 247, 1937, pp. 185 to 188. Uber das Krystallisierte Vitamin D_4.	1-9,13-15,17-20,27-31,33-36

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
25 OCTOBER 1991	20 DEC 1991
International Searching Authority	Signature of Authorized Officer
ISA/US	Mukund J. Shah

Form PCT/ISA/210 (second sheet) (Rev. 11-87)

FURTHER INFORMATION CONTINUED FROM THE FIRST SHEET

Group V, Claims 25, 2nd process of preparing vitamin D_4.
Group VI, Claim 26, 3rd process method of preparing vitamin D_4.
Group VII, Claim 32, animal feed.